PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C12N 11/14, C03C 14/00	A1	(11) International Publication Number: WO 86/ 04088
		(43) International Publication Date: 17 July 1986 (17.07.86)

(21) International Application Number: PCT/SE85/00524

(22) International Filing Date: 16 December 1985 (16.12.85)

(31) Priority Application Number: 8500082-6

(32) Priority Date: 9 January 1985 (09.01.85)

(33) Priority Country: SE

(71)(72) Applicant and Inventor: EHRNFORD, Edgar, Lars, Martin [SE/SE]; Sånekullavägen 31, S-217 74 Malmö (SE).

(74) Agent: AWAPATENT AB; Box 5117, S-200 71 Malmö (SE).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: CARRIER FOR IMMOBILISING BIOLOGICALLY ACTIVE ORGANIC MATERIAL

(57) Abstract

A carrier for immobilising biologically active materials, such as enzymes and microorganisms. The carrier is characterised in that it consists of a porous body of joined-together particles of a comminuted porous sintered glass fibre matrix. Preferably, the particles are held together at the points of contact with one another by a carbonised organic binder. The particles of sintered glass fibre matrix which, generally, have an average diameter of about 0.005-5 cm, preferably about 0.005-0.05 cm, preferably have a density of 20-2000 kg/cm^3 and have been obtained from fibres having a diameter of 0.3-100 µm. The carbonised organic binder preferably is selected among polymeric binders, such as polyacrylates, polyvinyl acetates, and polyvinyl acetals.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GA**	Gabon	**MR**	Mauritania
AU	Australia	**GB**	United Kingdom	**MW**	Malawi
BB	Barbados	**HU**	Hungary	**NL**	Netherlands
BE	Belgium	**IT**	Italy	**NO**	Norway
BG	Bulgaria	**JP**	Japan	**RO**	Romania
BR	Brazil	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SN**	Senegal
CH	Switzerland	**LI**	Liechtenstein	**SU**	Soviet Union
CM	Cameroon	**LK**	Sri Lanka	**TD**	Chad
DE	Germany, Federal Republic of	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America
FI	Finland	**MG**	Madagascar		
FR	France	**ML**	Mali		

CARRIER FOR IMMOBILISING BIOLOGICALLY ACTIVE ORGANIC MATERIAL

The present invention relates to a carrier for immobilising biologically active organic material.

By biologically active organic material are meant, in the context of this invention, substances having some type of biological activity, such as enzymes and microorganisms, including bacteria, fungi, algæ, cells and the like.

Immobilising such biologically active material by somehow anchoring it to a carrier, for example by adsorption or chemical bonds, is previously known. Normally, the carrier is a solid material which is substantially inert to the active material and its reaction products. The carrier usually is in the form of relatively large grains readily separable from a fluid. The advantages of anchoring biologically active material are well known and obvious and imply, above all, that one can better economise on the expensive active material in that it can be more readily separated and recovered from a fluid.

The known carriers comprise a multiplicity of different materials, such as pumice, silica, ceramics, polymeric materials etc. Also glass is a known carrier material, primarily in the form of microporous glass spheres. The production of such glass spheres is relatively complicated and expensive. Furthermore, the pores and voids have a shape such that the bulk transport to the immobilised active material is determined by diffusion, and therefore the rate of the reaction concerned will not be optimal.

It should be mentioned in the context that the use of mineral wool filters in biological wastewater treatment is previously known from, for example, NO published patent application 147,639 and SE patent

application 7308380-0 which are concerned mainly with mineral wool mats for use in buildings, and for the purpose of the present invention these mats have a permeability which is too high and also unsatisfactory dimensional stability.

Filter bodies of glass fibres are also known within the art, but these are not sintered glass fibre matrices, and the filter body is held together by a polymeric binder, such as an acrylate resin. This structure is unsuitable for the use which is contemplated in the present invention because the polymeric binder may affect the biologically active material and also, in the production of dense compressed filter bodies, may flow out and block the pores so that a homogeneous and uniform porosity is not obtained.

As an example of the above-mentioned prior art technique, mention may be made of DE 2,443,502 which relates to a carrier of the type filter candle for enzyme immobilisation. The carrier can be built up either of helically wound material or of porous particulate material. The porous particulate material may either be sintered or held together by curing by means of a polymeric binder. At the high temperature required for sintering, the inner pores of the particles presumably are fused together and become inaccessible, and similarly the use of a cured polymeric binder will cause the surface of the particles to be covered by the polymeric binder, such that the pore openings are covered and the pores also in this instance become inaccessible.

Furthermore, it is previously known from U.S. Patent 4,404,291 and DE Patent 3,103,751 to use a porous sintered low-density material for, for example, high-temperature filtration, absorption of liquids and as a carrier for catalytically active materials. According to this prior art technique, a nonporous pulverulent starting material consisting of glass,

glass ceramics or conventional ceramic material is sintered to give the final porous sintered material. The porosity of the material is here constituted solely by the voids between the sintered powder particles.

The present invention aims at providing a novel carrier for immobilising biologically active material, said carrier being solid, inert, uniform and entirely open, and having excellent dimensional stability, a high inner total surface, homogeneous and controllable porosity, and a structure which is characterised by even and smoothly rounded inner surfaces.

This object is achieved in that the carrier according to the invention is a porous body consisting of joined-together particles of a porous sintered glass fibre matrix. The particles are preferably held together at their points of contact with one another by means of a bond obtained in connection with the carbonisation of an organic binder.

Further characteristic features of the invention will appear from the appended claims.

Between the joined-together particles, a channel system is formed which permits liquid flow and, thus, good liquid contact with the individual particles. The inner pore walls of the particles are formed by solid glass fibre "rods" which have a well-defined diameter and are sintered together at the points of contact. Such sintering gives a glass fibre matrix having a three-dimensional lattice or network structure with even, homogeneous and smoothly rounded inner surfaces, which structure provides high strength and rigidity and is highly useful for the transport of substrates and products. Moreover, the glass fibre matrix is characterised in that it can be formed with extremely fine pores and still retain its open pore structure, which is not possible with other methods of making porous glass. For example, it may be mentioned that, with the methods most frequently used, the pores

are given the form of spherical voids interconnected by constricted apertures. According to another production principle, the pores form comparatively closed channels which are obtained by selective acid dissolution of a phase in a multiphase gaseous mixture. With conventional powder sintering technique, a low volume proportion of pores is obtained. With fine pore dimensions, a considerable proportion of the pores will, besides, be of the closed type.

By a sintered glass fibre matrix is meant that the original glass fibres have been compressed under pressure at elevated temperature, such that the fibres at their points of contact are fused together to provide a uniform matrix. The sintered glass fibre matrix forms a three-dimensional network with a regular open structure having high permeability to gas and liquid. Blocks consisting of the sintered glass fibre matrix are then comminuted into particles or disks.

Because the carrier according to the invention consists of joined-together porous particles, it provides, in a manner of speaking, a double porosity, i.e. both an "outer" porosity and an "inner" porosity. By outer porosity is here meant the ratio of the void volume formed between the joined-together particles to the total volume of the carrier, while the inner porosity is the ratio of the pore volume of the joined-together porous particles to the total volume thereof.

It is an important and significant fact that the carrier according to the invention exhibits, because of its composition, both an outer porosity and an inner porosity, the inner porosity being utilised primarily for safe and interference-free immobilisation of the biologically active material, such as an enzyme, while the outer porosity contributes to an effective contact between the biologically active material and the fluid upon which it is intended to act, thereby

to achieve an optimal reaction and an efficient removal of products formed.

Generally, the inner porosity of the sintered glass fibre matrix particles is determined by the density of the matrix and the diameter of the fibres employed. At a density corresponding to the one of the glass employed, there is obtained an entirely compact and nonporous matrix which lies outside the scope of the present invention. A general rule is that the particles in the carrier according to the invention have a density within the range 20-2000 kg/m^3, preferably 300-2000 kg/m^3, a density in the range 1500-2000 kg/m^3 being especially preferred.

The average diameter of the glass fibres utilised in the production of the sintered particles for the carrier according to the invention generally is within the range 0.3-100 µm, preferably 0.5-5 µm, a fibre diameter of 0.5-3 µm being especially preferred. A general rule is that the smaller fibre diameters make it possible to obtain particles with fine pores, simultaneously as the favourable three-dimensional network structure is maintained. In this respect, a fibre diameter below about 5 µm is especially advantageous. In the context of this invention, particles having fine pores and an open network structure are preferred because they are especially well suited for the immobilisation of biologically active material of small dimensions, such as enzymes and bacteria, simultaneously as an efficient transport of substrate and products is made possible.

As has been indicated above, the pore size of the sintered particles in the carrier according to the invention is determined by the density of the particles and the diameter of the fibres utilised in the production of the particles. By suitable selection of these parameters, it is thus possible to obtain the desired pore size of the particles. The par-

ticles according to the invention suitably have a pore size of at most about 20 μm, usually about 1-20 μm. A pore size within the range 1-15 μm is preferred, a pore size of about 5-10 μm being especially preferred. For the sake of clearness, it is pointed out that by the expression "pore size" is meant the diameter of a circular pore having the same cross-sectional area as the pores of the sintered particles. Furthermore, the pore size indicated is the average value of all pores in the particles. It should be noted, however, that the deviation from this average value of the pore size of the individual pores is fairly insignificant if fibres of the same diameter are used in the production of the particles.

As has been mentioned before, the particles in the carrier according to the invention consist of a sintered glass fibre matrix. The composition of the glass employed is not critical, although the glass must, of course, be a glass that can be converted into fibres. Thus, it is possible, within the scope of the invention, to vary the glass composition of the particles in many ways.

Even though the form of the porous sintered glass fibre matrix is not critical and may be varied in innumerable ways, it is preferred to produce it originally in the form of a planar disk. A simple method of making such a disk is to sinter the glass fibre matrix between two press plates at elevated pressure and temperature. The finished disk may have optional thickness, but preferably the thickness lies within the range 0.05-5 cm, most preferably 0.05-2 cm. The finished disk is then comminuted into fragments or regular pieces having an average diameter of about 0.005-5 cm, preferably 0.005-0.05 cm. By "average diameter" is here meant the diameter of a sphere having the same volume as the fragment or piece concerned. The fragments may also be in the form of disks having

a thickness of 0.02-0.5 cm, in which case the diameter is the average disk width.

After sintering into blocks, the glass fibre matrix thus is comminuted into particles, each of which consists of a three-dimensional network structure by which high mechanical strength is imparted to the particles which thus obtain good tolerance to being stacked in columns or subjected to strain during mechanical stirring. Before their use for the object of the present invention, the particles are joined together to form a three-dimensional network structure in connection with carbonisation of an organic binder. This is preferably carried out in such a manner that the glass fibre matrix particles are loosely combined to form the desired structure, for example in a mould, whereupon the organic binder is added, for example in the form of a liquid monomer for the organic binder or, if a thermoplastic binder is used, in the form of a molten binder. The structure is then impregnated either with a binder which then is caused to solidify, or with a binder monomer which is polymerised, and in this manner a solid self-supporting structure is obtained which is then heated, preferably in air, for "carbonisation" of the organic binder which thus is substantially completely removed from the free particle surfaces of the structure but imparts a bonding effect to the adjacent or adjoining contact surfaces of the particles, such that the initially loosely combined structure forms a firmly held-together unit with an open structure consisting of joined-together particles of a porous sintered glass fibre matrix, the particles being held together at their points of contact by a carbonised organic binder. Although the entire structure has been soaked with the organic binder, the latter is substantially completely removed from the free particle surfaces upon carbonisation, and the organic binder does not clog the particle

pores. In this manner, the finished carrier will have the above-mentioned advantageous combination of outer and inner porosity.

The organic binder employed in the context of this invention preferably is an organic polymeric binder. Examples of preferred polymeric binders are, for example, acrylate plastics, such as polymethacrylate and polymethyl methacrylate, including monomers to form these polymers; vinyl acetate plastic, such as polyvinyl acetate; and vinyl acetal plastic, such as polyvinyl acetal and polyvinyl butyral, but it should be noted that the invention is not restricted to these polymeric binders.

Highly complicated shapes (tubes, spirals etc.) are obtainable by an alternative and comparatively simple production method, according to which the particles are first impregnated with a highly viscous liquid polymer (such as urethan dimethacrylate or the reaction product of bisphenol-A and glycidyl methacrylate (BIS-GMA)) which cause the particles to stick together. The particle mass can now be given the desired shape which may be fixed initially by curing at least the superficially located polymer. This can be done for example by heating or irradiation with UV light or, alternatively, visible light. The method of curing is decided by the initiators and accelerators that have been added according to prior art technique. The body thus shaped and, possibly, fixed is then heated, optionally after embedding it in a refractory material, to a temperature sufficiently high to cause the particles to adhere to each other at their points of contact.

The carbonisation conditions for the polymeric binder are not critical, except that the temperature must not be so high that the softening point of the glass fibre matrix particles is exceeded and the particles soften and their pores collapse. During carbo-

nisation of the binder, the temperature must always be maintained sufficiently low to ensure that the pores of the glass fibre matrix particles will be kept intact. Generally, a suitable temperature range is about 500-700°C, preferably about 550-650°C, about 600°C being the temperature which is especially preferred at present.

The carbonisation atmosphere is not critical and preferably is air.

The carbonisation time must be sufficiently long to effect complete carbonisation of the organic binder, and generally a time of about 15-60 min. is satisfactory. In most cases, a time of about 30 min. is sufficient.

As has been mentioned before, the porosity of the glass fibre matrix particles, i.e. the inner porosity of the carrier, may be controlled within wide limits by controlling the fibre diameter and the sintering pressure. Similarly, the outer porosity of the carrier may be controlled by suitable selection of the dimensions of the glass fibre matrix particles. As also mentioned before, the sintered porous glass fibre matrix is comminuted into particles having an average diameter of about 0.005-5 cm, preferably about 0.005-0.05 cm. Normally, it is preferred that the particles have a relatively narrow particle size distribution, i.e. all particles have substantially the same average diameter, thereby to provide a carrier of substantially homogeneous outer porosity. However, there is no obstacle to mixing, if desired, particles with different average diameters, thereby to provide a carrier of varying outer porosity. The carrier may also be built up by different layers of different particle sizes, such that the outer porosity will be essentially homogeneous in each individual layer but vary from one layer to another.

As has been mentioned above, the carrier preferably is produced in that the glass fibre matrix particles are loosely joined together in a mould, whereupon the organic binder is added. Using a mould also makes it possible to vary the shape of the carrier in many different ways, such as disk shape, spherical shape, cylindrical shape, cubic shape, or the like.

When the carrier according to the invention is to be used, the biologically active material concerned, for example an enzyme, is first immobilised in the carrier, for example by being impregnated with a solution or dispersion of the active material. If this is a microorganism suspension, it will be sucked up as if by a sponge and can then be developed by the formation of micro-colonies in the network structure. The carrier with the immobilised active material is then ready for use, for example for carrying out an enzyme-catalysed chemical reaction. The carrier may be in the form of an aggregate or lumps which simply are introduced into the reaction medium which may consist of a substrate solution for the enzyme. When the carrier is brought into contact with the reaction medium, the reaction starts, and to facilitate and accelerate the reaction, the reaction medium suitably is stirred. At the end of the reaction, or when the reaction has advanced to the desired stage, the carrier is separated from the reaction medium by filtration or simply by removing the carrier lumps from the reaction medium.

In another type of application of the carrier according to the invention, the carrier is in the form of a disk or plate. The thickness of the carrier disk may be varied as desired, but preferably lies within the range 0.05-2 cm. In other respects, the carrier disk may have optional form, such as circular, elliptical or polygonal, for example rectangular or square. In a manner similar to what has been described

above, the biologically active material is first immobilised in the carrier disk, for example by impregnating the disk with a solution or dispersion of the active material, whereupon the carrier disk is ready for use, for example for analytical purposes. The fluid which is to be affected by the immobilised biologically active material is then contacted with the carrier disk, for example by letting the fluid flow through the disk. An example of this is a substrate solution flowing through a carrier disk in which an enzyme is immobilised. The reaction may be carried out in batches, but also continuously in that the carrier disk is inserted across the cross-sectional area of a tubular reactor so that the reagent fluid (such as a substrate solution) passes the thickness of the disk. With a suitable combination of excess liquid pressure and density of the carrier material, considerable amounts of liquid may be forced through the carrier. If the reagent fluid is to be subjected to several reactions after one another, such as several successive enzymatic reactions, this can be carried out very simply by providing several carrier disks at suitable intervals after one another in the tubular reactor, each individual carrier disk being provided with immobilised active material (enzyme) for the respective reaction. In this manner, highly compact and efficient biotechnical systems can be established.

It will be appreciated from the above description that the present invention provides a carrier intended for the immobilisation of biologically active material and having highly desirable and advantageous properties, such as great dimensional stability and strength, an open grid-like structure, and having a well-defined and reproducible, uniform porosity and pore size. Furthermore, the carrier is inert to the biologically active materials concerned and their reaction products, while at the same time having affinity to the immobili-

sation of biologically active materials.

The glass surface may be given positive groups by silanisation. The glass composition may vary, and tracer elements may be incorporated which, by ion exchange with the surroundings, can affect the conditions of life of the microorganisms. Other advantages of the glass structure are its hydrophilic character (facilitates substrate absorption etc.) and its excellent mechanical and thermal properties. The excellent mechanical properties protect, for example, accommodated cells during stirring or stacking in a column. Furthermore, the carrier is not decomposed under the action of cells which are being divided, or the action of an inner pressure during gas-forming reactions. The thermal stability provides for hardiness against high process temperatures and sterilisability. The method as referred to above for producing porous glass in the form of a three-dimensional network structure has the advantage that it gives an entirely open structure having a high pore volume and large total inner surface, which structure does not contain spherical and wholly or partly closed voids. In this manner, optimal conditions for substrate and product transport through the carrier medium are established. Provided that the fibres have a diameter of less than 5 μm, this method also makes it possible to produce extremely fine-pore (fine-mesh) structures with pores having an average diameter of less than 10 μm, while retaining the above-mentioned advantages. This is not possible with any other method previously disclosed. From the viewpoint of efficiency, it is especially desirable that the pore size be about 5 times the cell size, but not greater than 10 times the said cell size. In view hereof, fine-pore structures having pore sizes of less than 10 μm, in particular structures with pores within the range 3-10 μm, are especially desirable as carriers of microorganisms. On the other hand,

pore sizes of less than 3 μm are optimal in connection with enzyme processes, while pore sizes greater than 10 μm are desirable for the cultivation of, for example, mammal cells.

CLAIMS

1. A carrier for immobilising biologically active organic material, characterised in that it consists of a porous body consisting of joined-together particles of a porous, sintered glass fibre matrix, and that said particles are preferably held together at their points of contact with one another by means of a bond obtained in connection with the carbonisation of an organic binder.

2. A carrier as claimed in claim 1, characterised in that said particles have a density of 20-2000 kg/m^3.

3. A carrier as claimed in claim 1, characterised in that the sintered glass fibres of said particles have an original diameter of 0.3-100 µm.

4. A carrier as claimed in claim 1, characterised in that the average pore diameter of the porous particles does not exceed 20 µm.

5. A carrier as claimed in claim 1, characterised in that said porous particles have an average diameter of 0.005-5 cm, preferably 0.005-0.05 cm.

6. A carrier as claimed in claim 1, characterised in that said porous particles are in the form of disks having a thickness of 0.02-0.5 cm.

7. A carrier as claimed in claim 1, characterised in that said binder is a carbonised organic polymeric binder.

8. A carrier as claimed in claim 7, characterised in that said binder is a carbonised polyacrylate, polyvinyl acetate or polyvinyl acetal.

9. A carrier as claimed in claim 1, characterised in that said body consists of a disk having a thickness of 0.5-5 cm.

10. A carrier as claimed in claim 1, c h a r a c - t e r i s e d in that, within said body, the voids between the joined-together porous particles form an entirely open pore system.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE85/00524

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC [4]

C 12 N 11/14, C 03 C 14/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 2	C 07 G 7/00, /02-/028; C 12 D 13/10
IPC 3	C 03 C 19/06, /09, 14/00
IPC 4	C 07 K 17/14; C 12 N 11/00-/18; .../...

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	DE, C2, 2 443 502 (THE CARBORUNDUM CO, 14302 NIAGARA FALLS, N.Y, US) 13 March 1975 & FR, 2243011 GB, 1486305 CA, 1032882 JP, 50076286	1-10
Y	US, A, 4 404 291 (SCHOTT GLASWERKE, MAINZ FED REP OF GERMANY) 3 September 1983 & WO, 82/02707 DE, 3103749 EP, 0070849	1-10
Y	DE, C1, 3 305 854 (SCHOTT GLASWERKE, 6500 MAINZ, DE) 6 September 1984 & EP, 0117484 JP, 59156923	1-10

.../...

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1986-03-19	1986 -03- 25
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Yvonne Siösteen

L.E

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

II Fields searched (cont).

IPC 4 G 01 N 33/551-/552;
C 12 M 1/40

US Cl 65:21.4, 156;
435:174-182

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ..... because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	SE, B, 399 699 (JENAER GLASWERK SCHOTT & GEN., MAINZ, DE) 27 February 1978 & DE, 2424579 FR, 2272047 GB, 1479539 SE, 7505729	1-10
A	DE, A1, 2 707 024 (OWENS-ILLINOIS, INC-TOLEDO, OHIO) 22 September 1977	1-10
P	DE, A1, 3 410 650 (KERNFORSCHUNGSANLAGE JÜLICH GMBH, 5170 JÜLICH, DE, SCHOTT GLASWERKE, 6500 MAINZ, DE) 3 October 1985	1-10